EPA United States Environmental Protection Agency

Risk Assessment Evaluation for Concentrated Animal Feeding Operations

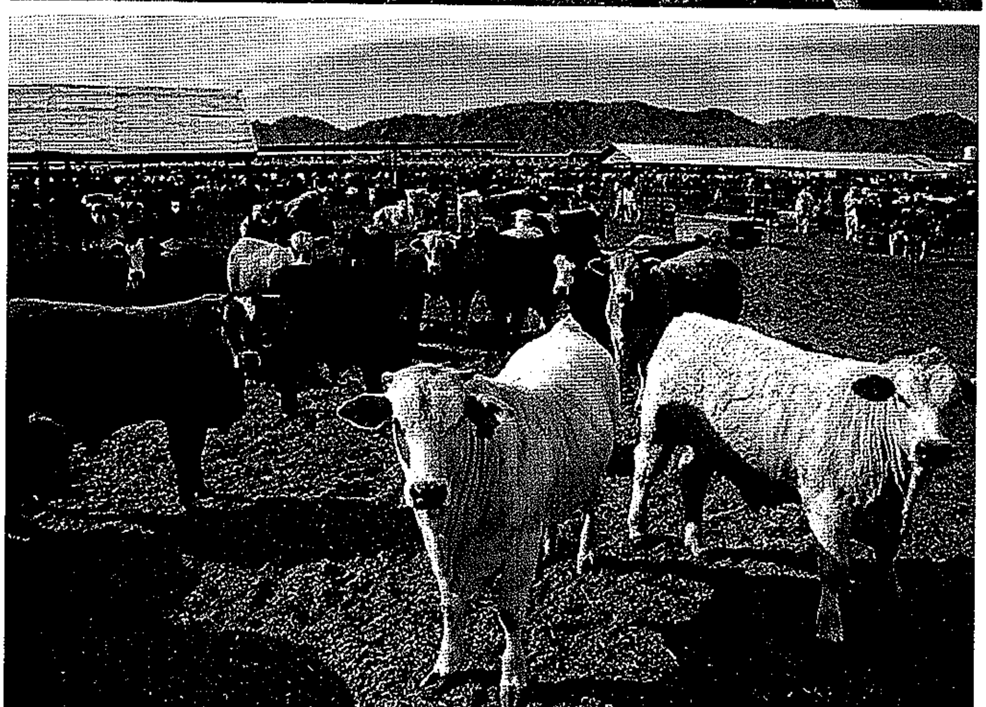

Exhibit # 7

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Risk Management Evaluation For Concentrated Animal Feeding Operations

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Abstract

The National Risk Management Research Laboratory (NRMRL) developed a Risk Management Evaluation (RME) to provide information to help plan research dealing with the environmental impact of concentrated animal feeding operations (CAFOs). Methods of animal production in the U.S. have undergone fundamental changes in the last 30 years. The majority of meat, dairy, and poultry production has been concentrated into large facilities. Dairies with more than 2,000 cows and swine operations with more than 10,000 hogs are not unusual. Broiler houses with 50,000 birds are common. With the concentration of animals has come a concomitant concentration of manure production. One animal facility with a large population of animals can easily equal a small city in terms of waste production. Current practices of waste handling often include minimal or no treatment before the wastes are disseminated into the environment. The RME was developed to provide characterization of the waste problem, and a description of common environmental stressors and their movement including the air transport of pollutants. Current risk management practices in the animal industry are described, along with treatment approaches such as anaerobic/aerobic digestion, constructed wetlands, and disturbed land reclamation. Finally, suggested areas for future research are presented to help focus planning for the near future.

1 OVERVIEW OF RISK MANAGEMENT DOCUMENT

This document is intended to help the reader gain an understanding of potential environmental problems associated with Concentrated Animal Feeding Operations (CAFOs). Although a variety of animals are raised in CAFOs, this document will focus on beef, dairy, swine and poultry. The quantities and characteristics of manure produced by the different animals are presented. The watershed stressors resulting from CAFO pollution are discussed, as are the transport mechanisms that disperse them through the environment. Common manure management practices are also presented.

Because large numbers of animals are confined in relatively small areas at CAFOs, a very large volume of manure is produced and must be kept in a correspondingly small area until disposed of. The age-old practice of land application is used, but the volumes of manure that must be disposed in this way frequently exceed the assimilative capacity of land within economic transport distances. This may result in the release of excess manure to watershed environments during the catastrophic breach of holding facilities or more commonly, during the intermittent runoff of excess manure applied to already saturated land. Figure 1.1 shows the phosphorus assimilative capacity of farmland in the United States. Figure 1.2 shows the excess phosphorus available on farms with no export. Clearly, an imbalance exists between available phosphorus and the capacity of the land to absorb phosphorus. The same general relationship holds for nitrogen. If land in entire counties were available for application of animal waste, the overburden of nutrients is somewhat relieved, but excess quantities of nutrients still exist in some locales. Neither of the maps shown takes into account fertilizer applied to fields.

This would be a problem even if manure contained only beneficial nutrients. In excess amounts, these nutrients damage, not improve, soil fertility and may pollute nearby water. More importantly, however, manure from CAFOs contains components other than nutrients. The dominant element in manure is carbon. Many of the carbon compounds in manure may contribute to oxygen depletion in water. The nutrient elements N and P in manures may also contribute to eutrophication of water if their entry into water is not controlled. Modern agriculture with its emphasis on intensive housing and speeding the growth of livestock to market weight has employed a variety of substances that have not been used before in animal husbandry. These include antibiotics to combat the spread of disease among animals housed in close quarters, natural and synthetic hormones to speed growth, and metals (As, Cu, Zn) to do the same and preserve the freshness of feed. When present in the large amounts of manure generated at CAFOs and stored on-site, these other substances pose a threat to the environment. The effects of antibiotics on native soil bacteria are largely unknown. The effects of biogenic and synthetic hormones on other animals and humans are largely unknown.

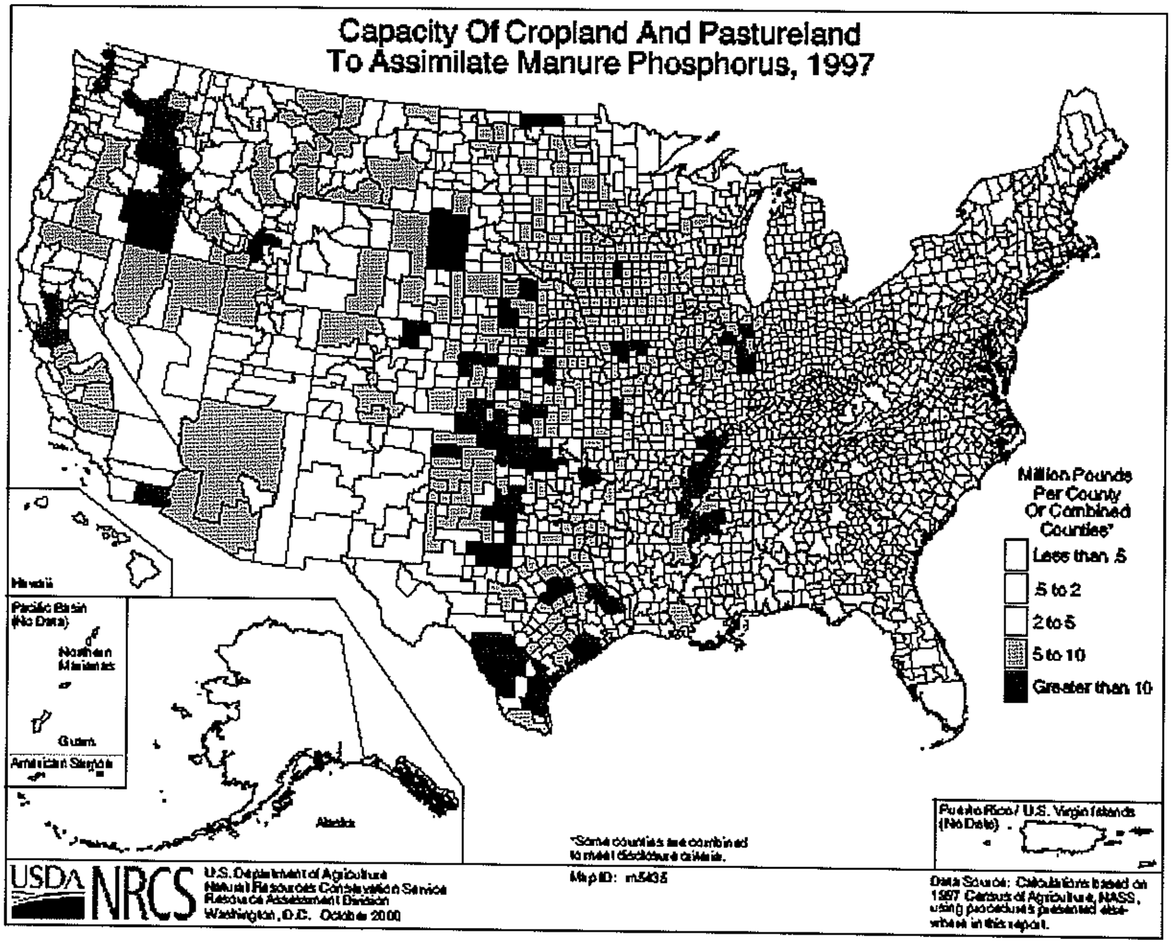

Figure 1.1. Phosphorus assimilative capacity for farms.

This Risk Management Evaluation (RME) is intended to document the salient environmental risks associated with hog, poultry, dairy and beef CAFOs and actions that could be taken to reduce those risks now. Areas in which further research is needed are identified and discussed in Section 8 of this document.

In reviewing the existing body of knowledge on intensive livestock agriculture, the following points became clear.

- Underlying all of the environmental problems associated with CAFOs is the fact that too much manure accumulates in restricted areas. Traditional means of using manure are not adequate to contend with the large volumes present at CAFOs.

- The nutrient load from CAFOs is large, with about 2.5 billion pounds of N and 1.4 billion pounds of P recoverable in manure. Total manure N is about 12.9 billion pounds and total manure P is about 3.8 billion pounds.

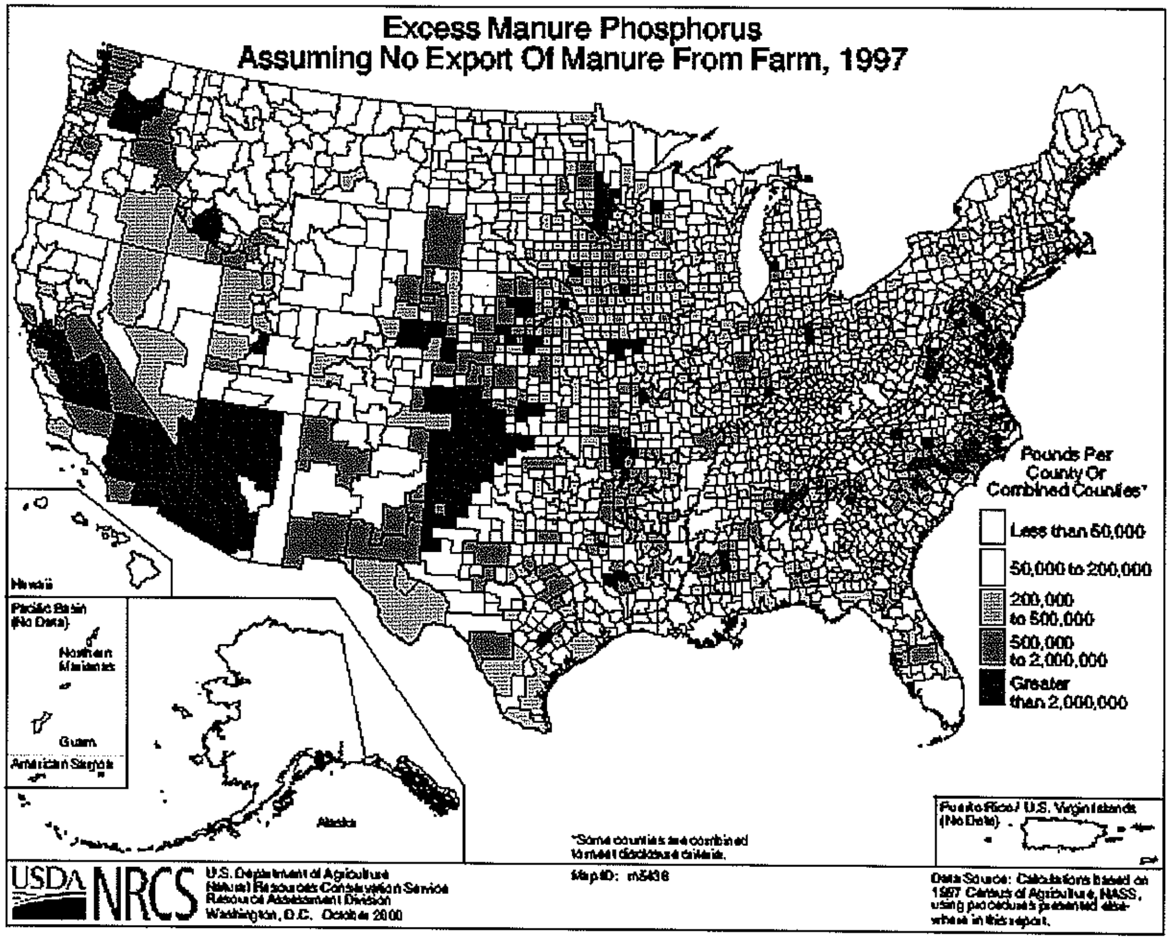

Figure 1.2. Excess phosphorus on farms with no export.

- CAFO manure contains potentially pathogenic microorganisms. The combination of large herds and closely confined housing makes it likely that at least some animals are asymptomatic carriers of pathogenic organisms. Once introduced, these pathogens may readily spread among the closely confined herd. Shed into the manure, these pathogens find favorable breeding grounds in the barns, manure storage and handling systems and are released into the watershed environment routinely during the land application of waste.

- The antibiotics administered to CAFO livestock may contribute to the development of antibiotic resistant strains of pathogens – especially those harbored within the livestock raised at these facilities. The sub-therapeutic use of antibiotics at CAFOs aggravates the problem.

- Naturally occurring and synthetic hormones administered to livestock to speed growth to market weight pollute the environment when released along with manure during land application or during an accidental release. The environmental effects of these compounds are largely unknown.

- Metals used as feed supplements to promote livestock growth may degrade the quality of the land to which waste is applied. Adverse environmental effects may result when waste containing metals is released into the watershed.

- Transport pathways for stressors from CAFOs encompass surface runoff, air transport and redeposition, and groundwater flow. Nutrients, pathogenic organisms, hormones and metals may easily reach waterbodies via these means.

There are measures that may be taken now to mitigate the risk posed by the large volumes of manure at CAFOs.

- Reduce the volumes of manure created by changing waste management, handling practices, and feed utilization efficiency.

- Treat manure to kill pathogens, attenuate hormones and other organic contaminants, and stabilize metals.

- Increase use of anaerobic treatment and composting to control odors, nutrients, pathogens, and generate renewable energy.

- Reduce the use of antibiotics to stem the development of antibiotic resistant pathogens.

- Increase soil conservation methods to reduce runoff and erosion from fields to which manure has been applied. Reduced tillage, terraces, grassed waterways, and contour planting offer conservation benefits.

- Install barriers such as riparian zones and wetlands to prevent manure-laden runoff from fields from reaching streams.

- Change barn ventilation and manure management and handling practices to minimize the airborne release of stressors.

- Where economic factors work against making changes to CAFO management practices, eliminate them or provide incentives for making such changes.

Additional research needs to be undertaken to develop a range of alternatives for managing CAFO manure. The U.S. Department of Agriculture is engaged in research to address many of these questions, especially with respect to nutrient issues. EPA intends to complement their efforts by working with them on mitigation strategies for nutrients and, more importantly, focusing on pathogen, hormone and metal issues.

The environmental challenges posed by CAFOs are not insoluble. In some cases, simple management of wastes in different ways will ameliorate some of the problems. More attention to good soil management and application of wastes at phosphorus based agronomic rates will reduce loads of pollutants reaching water bodies. Development of means to extract value from wastes will be needed to make treatment feasible and reduce health risks. Nitrogen, phosphorus and methane are some of the potentially valuable products recoverable from manures. The key problem for managing CAFO waste is one of distribution of the manure from points of production to application sites in an economically viable manner.

Beyond manure management, new issues are emerging such as the environmental impact of aquaculture and other intensive agricultural operations, the environmental effects of different types of mortality management, and how to mitigate the hydrologic changes brought about by large CAFO operations. These issues will be addressed in future versions of this RME.

4 WATERSHED STRESSORS IN CAFO WASTE

The pollutants potentially leaving the CAFOs may affect watersheds directly or indirectly. The most often cited stressors affecting watersheds include nutrients, pathogens, sediments, EDCs, antibiotics, and metals. Direct effects occur when wastes flow directly into a receiving water as a result of poor storm water management or catastrophic failure of containment facilities. Indirect effects occur when wastes have been applied to a field and are subsequently moved into waterbodies by runoff after rainfall, percolation into groundwater with subsequent entry into streams or tile drain lines, wind driven movement, or volatilization and redeposition as in the case of ammonia.

The nutrient content of the manure generated on the CAFO is one of the most significant problems. Nitrogen in the waste may be transferred in the environment two ways. Ammonia may be volatilized from the waste directly into the air and generate odor and downwind deposition problems. Nitrate generated in the soil applied waste may enter surface or groundwater and may exceed the national drinking water limit of 10 mg/L to cause health problems in young children.

Phosphorus in waste may easily exceed crop requirements for a given year on a localized basis. If continual applications are made year after year, the soil becomes saturated with P and the potential for runoff losses and groundwater losses greatly increase.

The soil, if eroded will contribute to stream degradation by eutrophication. Erosion of soil onto which manure has been applied, may contribute to other environmental problems in waterbodies. Organic matter exerts an oxygen demand leading to a depression of dissolved oxygen. Solids, as either manure particles or eroded soil particles, increase the sediment load in streams and may unduly shade some parts of the stream. Other habitat effects will be associated with increased sediment load.

Microorganisms associated with manure may present a significant risk to health. The population of several known pathogens may be quite high in manure. Runoff from land application sites may carry large numbers of organisms into streams. Recreational use of the streams may then bring people into direct exposure to large numbers of potentially pathogenic microorganisms. Several disease outbreaks have been associated with manure contamination of water or food that has been contacted by manure.

There are also concerns associated with the potential metal content of poultry or swine waste. Trace levels of arsenic are added to poultry feed to promote growth. Similarly, copper is added to swine feed for growth promotion. Antibiotics, hormone compounds, and pesticides are found in animal wastes, and the environmental effects of these compounds are largely unknown. The following sections are meant to summarize the most pertinent literature concerning nutrients and other stressors from CAFO manure. The literature in the area of nutrients and nutrients as pollutants is overwhelming. This is an attempt to limit the literature review to the citations that have the most impact on EPA's mission.

4.1 Nutrients

"Livestock wastes, which for present purposes are defined as liquid and solid excreta with the associated remains of bedding and feed and sometimes with water added, have long been ranked among the farmer's most valuable resources. For traditionally, the fertility of his land has depended in very large measure on the supply of such waste, sometimes dropped in his field by grazing animals or sometimes stabilized in the steading into farmyard manure by the addition of straw. In the days of the agricultural revolution the efficiency of the yards as a '*manure factory*' was one of the primary criteria of farmstead design. More recently and more drastically, a variety of agricultural changes have combined to convert, under certain circumstances, this potential asset (manure) into an increasing liability. The agricultural changes result from growing economic pressures to increase the animal outputs by an increase in the number of livestock carried per unit of land." (ARC 1976)

4.1.1 Nitrogen

Animal waste contains nitrogen in organic and inorganic forms. The inorganic form is ammonia, and organic forms include urea and an array of organic compounds. Nitrogen compounds may move in a watershed in air, surface runoff, or through percolating groundwater. Any form of nitrogen may have an impact on a watershed because it is a major plant nutrient. Ammonia is immediately available to plants as ammonium ion. Ammonia may move as an air pollutant after volatilization from animal waste. In the soil, ammonia enters solution as ammonium ion that may be held on soil colloid exchange sites. Ammonium is formed when organic-N such as urea is metabolized either aerobically or anaerobically to NH_3 that ionizes in water to ammonium. Ammonia may lead to eutrophication, excessive oxygen demand in surface waters and fish kills, reduced biodiversity, objectionable tastes and odors, and growth of toxic organisms. Both forms of ammonia, NH_3 and NH_4^+, are toxic to aquatic life, although NH_3 is more toxic to fish. Ammonia may be converted by nitrification to nitrite and nitrate. Nitrite is toxic to fish and most aquatic species. Nitrite does not accumulate in the environment because it is rapidly oxidized to nitrate naturally by aerobic bacteria. Nitrate is highly mobile and may easily leach downward through the soil profile to an aquifer. Nitrate is the most widespread agricultural contaminant in drinking water wells (U.S.EPA, 1998). A drinking water maximum contamination level (MCL) of 10 mg/L has been set for nitrate-N based upon its role in the "blue baby syndrome" or methemoglobinemia. Nitrate may be converted to nitrite by nitrate reducing bacteria found in the low acidity infant stomach. Nitrite may then attaches to fetal hemoglobin in human infants forming methemoglobin, which is ineffective as an oxygen carrier. This toxicity, if not treated, may be fatal (Goldstein et al., 1974). Figure 4.1 depicts processes primarily responsible for transformation of nitrogen compounds in sediments at the bottom of lagoons (collection ponds) or in a topsoil layer treated with animal manure.

Soil profile characteristics and management practices may significantly affect leaching of nitrate and ammonium in feedlots and crop fields (Saint-Fort et al., 1995). Whereas runoff is the primary mechanism for the transport of sediment bound and solution phase ammonium, groundwater flow is the primary contributor of nitrate to surface water from agriculture. (Follet, 1995). Spatial variability of nitrate in ground water and temporal fluctuation are related to seasonal recharge and hydrologic variations in the region (Halberg, 1986). High concentrations of nitrate in groundwater are associated with high permeability soil and aquifer material, such as permeable sand and gravel, karst limestone, or fractured rock (Hitt et al., 1999). In these landscapes, manure applied as fertilizer is susceptible to relatively rapid infiltration, thus contaminating ground water with nitrogen and/or phosphorus.

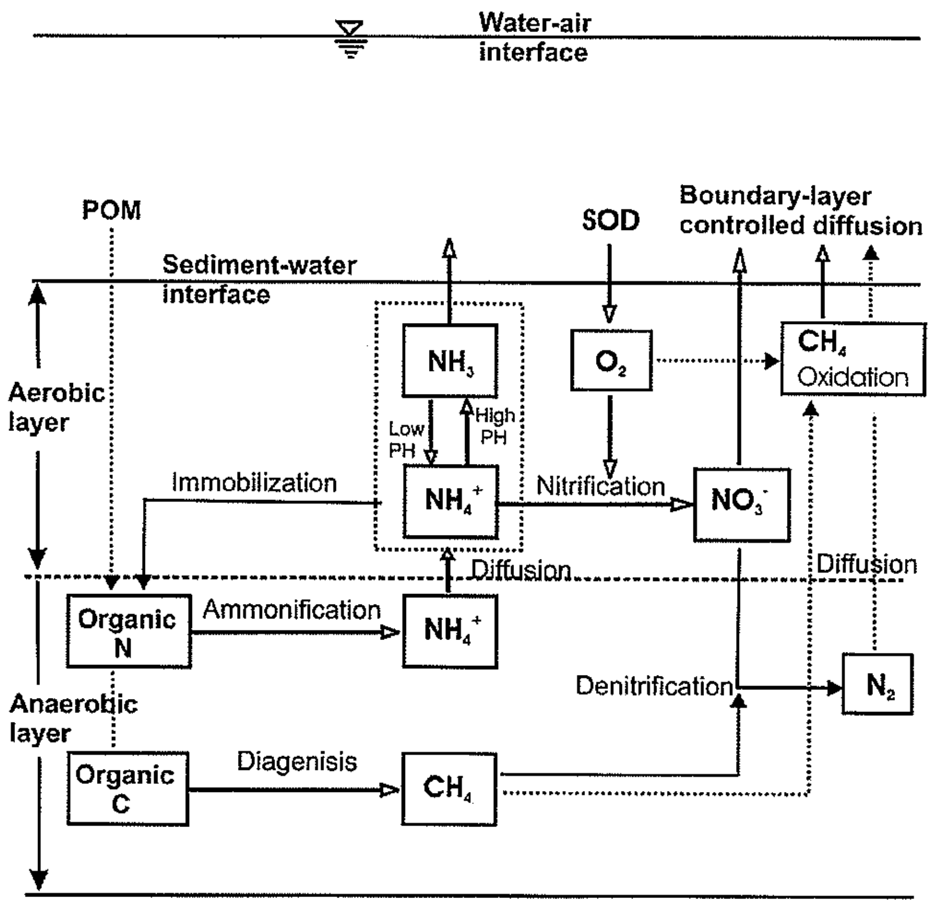

Figure 4.1. Depiction of carbon and nitrogen cycles in soils or sediments.

Leaky lagoons and below grade storage facilities are potential sources of nitrogen compounds that may enter groundwater. As structures age, the integrity of the walls and bottoms of the lagoon may be penetrated by burrowing animals, or the lagoon walls and bottoms may develop cracks from wetting and drying cycles as the water level in the lagoon changes (U.S. EPA, 2001). Rupture of lagoon seals may be attributed to drying of exposed embankments when lagoon levels drop or gas release from microbial activity in soil beneath the seal (Ciravolo et al., 1979: Parker et al., 1999). Short-circuits to natural filtering, such as uncapped or improperly capped wells and infiltration in vegetated filter strips adjacent to lagoons are potential sources of groundwater contamination (U.S. EPA, 2001). Groundwaters in areas of sandy soil, karst formations, or sinkholes are particularly vulnerable to nitrogen infiltration. Leaching of ammonia compounds is generally not a significant transport mechanism, because ammonium may be sorbed to soils, fixed by clay minerals and organic matter, or transformed into organic forms by soil microorganisms through the process of immobilization (Follet,1995). Mineralization is a process whereby organically bound nitrogen is converted to inorganic mineral forms, (NH_4^+ and NO_3^-). Legume crops may fix atmospheric nitrogen by transforming (N_2) to ammonia. Ammonium adsorbed onto soil below liners in abandoned dry lagoons, through nitrification, may produce nitrate (Ham, 1999) that is potentially available for leaching into the deep subsoil and ground water. Two modes may dominate transport of pollutants in soils: 1) rapid advection through macropores; and 2) slow percolation through the soil matrix. The first transport mode, which is promoted by gravitational forces through macro-channels, is also referred to as preferential flow (Figure 4.2). The second mode is much slower and is governed by gravity drainage and capillary forces at

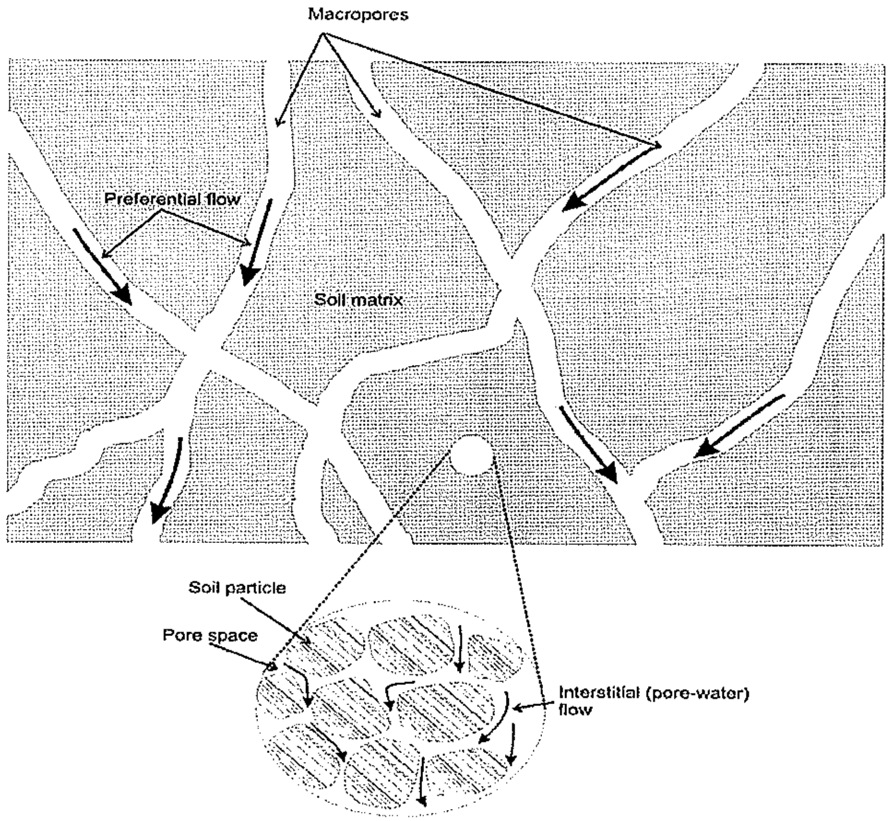

Figure 4.2. Diagrammatic illustration of preferential flow through macropores and interstitial (pore-water) flow in the soil matrix.

work through interstitial pore space. Preferential flow through macropores in soils beneath a waste lagoon may transport NH_4^+ or nitrate to ground water. Subsurface runoff and tile drainage are other transport pathways for nitrogen to surface waters.

Percolating water and leachate below lagoons may transport nitrate to ground water. Preferential flow through macropores and karst formations are also transport pathways to ground water. In heavily tile-drained watersheds most of the N added to surface water originates from tile drainage (Kovacic et al., 2000). In some areas nearly half of the applied fertilizer nitrogen may be discharged with tile-drainage water (Kanwar et al., 1983).

Nitrogen retention in the soil by adsorption of NH_4^+ onto soil colloids may constitute a source of NO_3^- to ground water (Ham, 1999). Urea and organic forms of N are also susceptible to leaching to ground water. Under anaerobic conditions, nitrate may be reduced to N_2 by denitrification, a primary process in reducing nitrate in ground water (Crandall, 1999). Denitrification occurs in the absence of dissolved oxygen and in the presence of chemically reduced compounds such as organic carbon or some divalent metals.

4.1.2 Phosphorus

Phosphorus exists as both organic and inorganic forms in animal waste. Inorganic phosphate in manure is easily adsorbed to soil particles, and thus has limited leaching potential. Organic P compounds are generally water soluble and subject to leaching (Sweeten, 1991).

Organic phosphate may easily be metabolized to inorganic phosphate that is the form that is useful as a nutrient. Inorganic phosphate in surface water is a major contributor to eutrophication. Because most surface water plant and algal growth is rate limited by phosphate level, pollutant phosphate is of particular concern. In concentrations over 1.0 mg/L phosphate may inhibit floc formation in drinking water treatment plants (Bartenhagen et al., 1994).

Phosphorus is much less susceptible to leaching because of its adsorption onto soil particles and therefore, poses less of a threat to groundwater than nitrate. Adsorption-desorption reactions in the soil regulate the rate at which P may be released (Siddique et al., 2000). Phosphorus accumulation in topsoil from animal waste and fertilizers constitutes a sediment problem more than a groundwater problem because P binds to the most erodible soil components (clay, organic matter, and oxides of Fe and Al)(Sims et al., 1998). However, if continual applications are made year after year, the soil becomes saturated with P and the potential for runoff losses and groundwater losses increases greatly. Phosphorus leaching may occur in sand soils where over-fertilization and/or excessive use of organic waste have increased soil P levels in excess of crop requirements (Sims et al., 1998). Preferential flow through macropores (e.g. soil cracks, root channels, earthworm borrowings) may transport a significant part of the phosphorus by suspended soil material to tile drains (Øygarden et al., 1977). Leaking from lagoons is also a likely source for groundwater contamination by phosphorus.

Environmentally significant export of anthropogenic P from agricultural soils by subsurface runoff begins with downward movement of P, either by slow leaching through the soil profile or preferential flow through macropores (e.g., soil cracks, root channels, earthworm borings). Dissolved inorganic P concentrations in subsurface runoff in artificial drainage systems may be higher than values associated with eutrophication of surface waters (Ryden et al., 1973, and Sims et al., 1998). P leaching may occur in deep sand soils, in high organic matter soils, and soils where over-fertilization and/or excessive use of organic waste have increased soil P values well above those required by crops. Leaching potential of P increases in soils with low concentrations of soil constituents that are primarily responsible for P retention, such as clays, oxides of Fe and Al, and carbonates (Sims et al., 1998). Mineralization of organic P and preferential flow through macropores and cracks caused by conservation tillage systems increase P concentration in drainage waters, including sediment-bound P.

4.1.3 Mineral Salts

Mineral salts of major concern in animal waste include the cations sodium, calcium, magnesium, and potassium and the anions: chloride, sulfate, bicarbonate, carbonate, and nitrate. These mineral salts, when applied repeatedly, may accumulate and increase soil ionic strength to levels that are toxic to plants and animals. Runoff may contribute to surface water salinization and leaching salts may affect ground water quality. Trace elements such as arsenic, copper, selenium, and zinc are often added to animal feed as growth stimulants and biocides. These when land applied may accumulate and adversely effect both human and ecologic health.

4.2 Pathogens

Animal manure is a potential source of pathogens. The organisms of concern in animal waste may be bacteria, fungi, protozoa, viruses, or worms. When released into the environment, these organisms may adversely effect human and animal populations. Although CAFOs are not the only source of these microorganisms, they are a major source of pathogenic contamination in most watersheds (Pell, 1997). Indeed, of the water bodies evaluated by the states, as required by the Clean Water Act, 36% of rivers were unfit for swimming and/or fishing as the result of pathogenic contamination largely attributed to CAFO operations (USEPA, 2001). In addition, the source waters from which drinking water is obtained for up to 43% of the United States comes from waters that are impaired by pathogenic contamination from CAFO operations (USEPA, 2001). About 15% of the population of the United States obtains drinking water from individual wells. When wells are located in areas hydrologically connected to CAFO operations, individuals using these wells may be exposed to pathogenic organisms present in the groundwater. Without purification, this may result in illness. CAFOs are likely to release pathogens into the environment for several reasons. First, because of the large number of animals kept in CAFO operations, the likelihood that one or more of the animals is infected with one or more pathogens is very high (Clinton, et al. 1979, Pell, 1997, Wesley et al. 2000). Second, because of the large volume of waste produced, manure may not be disposed of on-site in such a way that the pathogens will be killed or inactivated. Without treatment to reduce pathogen loads, storage and disposal practices will only serve to disseminate the microorganisms more widely in the environment.

Conventional water treatment is adequate to prevent the entry of bacterial contaminants into public drinking water supplies. Protozoan contaminants are usually in the form of cysts that are very resistant to chlorination. Drinking water treatment needs to be designed and operated properly to remove *Cryptosporidium* oocysts (Patania et al., 1995). Filtration through sand filters is usually necessary to remove protozoan cysts.

For the purpose of this RME only selected pathogenic organisms known to have a significant impact on human health or the environment and that are likely to come from CAFOs will be discussed. Before beginning a detailed discussion of these organisms, however, we will first discuss pathogenic organisms in general, their effects when released into the environment, and finally, relate the organisms to the CAFO species that is most likely the reservoir for each organism.

4.2.1 Pathogens of Concern at CAFOs

More than 130 microbial pathogens have been identified from all animal species that may be transmitted to humans by various routes (USDA, 1992; USEPA, 1998). Of these, 24 pathogens are likely to originate from animal populations. Historically, fewer than ten have caused significant disease outbreaks among humans. Potential environmental exposure to human populations extending beyond animal handlers exists for cryptosporidiosis, giardiasis, campylobacteriosis, salmonellosis, colibacillosis, leptospirosis, listeriosis, and yersiniosis; and many large-scale outbreaks have been attributed to each of these pathogens. Pathogens include bacteria, fungi, viruses, helminths (parasitic worms), and protozoa. Not all pathogens are present at every CAFO. Understanding the distribution of pathogenic organisms makes it easier to design strategies that will reduce risk. Table 4.1 lists commonly occurring diseases and the animals that are associated with these diseases. A general discussion of each of these classifications follows.

4.2.1.1 Bacteria

Bacteria are single-celled, prokaryotic microorganisms that are capable of causing disease in larger organisms, although most bacteria are non-pathogenic. They may grow and proliferate within higher organisms and are shed in feces. The presence of large volumes of feces in and around animals in CAFOs provides a breeding ground for many bacteria. The bacteria that have been shown to have the widest environmental impact when released into the watershed include *E.coli* 0157:H7, *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria*. The primary concern is that disease outbreaks may occur after

Table 4.1 Diseases and animals commonly identified as sources of the causative organisms.

	Hogs	Poultry		Cattle	
Disease		Turkeys	Layers	Beef	Dairy
Colibacillosis				*	*
Salmonellosis	*	*	*	*	*
Campylobacteriosis		*	*	*	*
Listeriosis				*	*
Yersiniosis	*				
Protozoa				*	*
Cryptosporidiosis				*	*
Giardiasis				*	*
Fungi					
Viruses	*	*	*	*	*
Helminths					
Endotoxins	*	*	*	*	*

contact with these organisms via swimming, eating shellfish, eating contaminated food, or drinking contaminated water.

4.2.1.2 Fungi

Fungi are either single celled organisms or multicelluar, eucaryotic organisms that may cause disease in other organisms. Fungal diseases are commonly difficult to treat and may persist for long periods of time. Common diseases include candidiasis, histoplasmosis, aspergillosis, and dermatomycosis.

4.2.1.3 Viruses

Viruses consist of nucleic acid molecules packed within a surrounding protein coat. Viruses only actively replicate when they have invaded a host cell. The virus genes take over the host cell metabolism to make more virus particles at the expense of the host cell. There is some evidence that reoviruses and many enteroviruses may be transmitted from animals to man. Also, a number of rotaviruses are known to cause diarrhea in both cattle and humans. Among farm workers, vesicular stomatitis is frequently transmitted from sheep to humans, and the potential spread of cow pox virus (vaccinia) to humans was the basis for the classical immunological practice of vaccination. Present day surveys indicate that rabies is more likely to be transmitted from cattle to man than from either cats or dogs. At this time much less specific information is known about the actual transmission of viral diseases from livestock to humans.

4.2.1.4 Helminths

Intestinal parasitic worms occupy space in the host organism's intestinal tract. The worms absorb nutrients from the host and thereby create a burden on the host. The prevalence of worms has declined in the United States. Transmission is frequently through oral-fecal routes or from exposure through food contaminated with manure.

4.2.1.5 Protozoa

Cryptosporidium parvum: Among humans cryptosporidiosis is caused by the protozoan parasite, and it has recently been determined that there are two separate genotypes, Type 1 (human) and Type 2 (bovine), that can cause human infections. For the Type 2 genotype, the infective dose may vary from 10 to

1000 oocysts and infection is generally more severe in children and immuno-compromised individuals. Virtually all cattle herds carry some level of cryptosporidiosis, and persistence and spread in the environment is aided by passive transfer from rodents and birds. Infected animals can shed more than one billion oocysts per gram of manure. Many large-scale waterborne outbreaks have occurred in the United States. Conventional drinking water disinfectants such as chlorine and chlorine dioxide are not effective in killing *C. parvum*. The standard water treatment processes of coagulation, flocculation, and filtration are thought to be effective in removing this parasite when operating normally.

Giardia lamblia: Giardiasis among humans may be traced to many possible sources including foodborne and waterborne transmission. It has been estimated that 2% of the population has been infected with this organism, and more outbreaks result from a waterborne origin than those caused by contaminated food sources. Wild animal populations such as deer, beavers, and bears may be the cause; however, more than 50% of dairy and beef cattle herds in the United States are infected with this organism. Infection may result from ingestion of only one oocyst, and once diarrhea occurs it may last up to two weeks. An ELISA assay for the detection of oocysts is readily available, and a vaccine for giardiasis is available for dogs and cats.

4.2.2 Disease Descriptions

Some of the diseases involved in significant waterborne disease outbreaks are summarized below.

Enterohaemorrhagic Colibacillosis (Escherichia coli (EHEC) O157:H7). There are many serotypes of *Escherichia coli* from animal sources that may infect humans. This group of diseases is referred to as colibacillosis. CAFOs, specifically cattle operations, may be sources of the organisms. However, among the various enteropathogenic and enterotoxigenic forms, *E. coli* O157:H7 clearly has the most serious manifestations. The hemorrhagic-toxigenic symptoms may often lead to death in 5-7% of infected individuals. The infective dose is thought to range between 10 and 1000 organisms. Contamination with cattle feces is known to be the most likely source of infection in the U. S. with foodborne infections ranking highest; however, waterborne and recreational exposure is also associated with this disease. Interestingly, outside of the United States isolation of cultures of *E. coli* O157: H7 is associated with sheep. Although swine and poultry carry many strains of *E. coli*, the specific Strain O157:H7 has not been isolated from these farm species. Three *E. coli* outbreaks (one in Montana in 1995, one in Illinois in 1996, and one in Connecticut in 1996) were traced to organic lettuce growers. It is suspected that the lettuce was contaminated by infected cow manure (Nelson, 1997).

Campylobacteriosis ***(Campylobacter jejuni)***: This organism is the leading cause of bacterial diarrhea in the United States, the most common source being chickens, or more correctly, fecal contamination of poultry meat. This organism is also commonly transmitted by cattle, birds, and even flies. While the digestive tract of chickens contains many species of *Campylobacter*, it appears that most human infections are caused by four thermophilic strains of this organism. *C. jejuni* causes a watery diarrhea that is only occasionally bloody. Other symptoms include fever, abdominal pain, nausea, headache, and muscle pain. The illness usually lasts two to five days, but reinfection is common and treatment with antibiotics (preferably erythromycin) is not usually necessary. Surveys show that 20-100% of retail chickens are contaminated. When human outbreaks occur they are usually small (less than 50 individuals) although one large outbreak (2,000 people) occurred in Bennington, VT in 1978. Guillain-Barre syndrome may occur as a sequel to this infection as well as meningitis, recurrent colitis, and acute cholecystitis, but these occurrences are rare. Although chickens are the primary animal species associated with this organism, transmission from infected milk is relatively common.

Yersiniosis (*Yersinia enterocolitica*): This organism is a gram-negative rod that is often isolated from wounds, feces, sputum, and mesenteric lymph nodes. CDC estimates that 17,000 cases occur annually in the U.S. It is one of the three most significant microbes than can originate from large swine operations. Yersiniosis is frequently characterized by diarrhea and/or vomiting, fever, and abdominal pain. Similar to Salmonellosis, postenteritis arthritic conditions occur in 2-3% of the affected individuals.

Listeriosis: The CDC estimates that approximately 1600 cases of listeriosis occur each year with 500 resulting in death. It is believed that cattle that are being fed silage are much more likely to harbor this organism. Two separate clinical disease patterns may follow infection with *Listeria monocytogenes*. The more mild form is commonly referred to as gastrointestinal listeriosis and is characterized by a rapid onset of diarrhea, abdominal cramps, and nausea. The more serious form of the disease is referred to as listeriosis. Symptoms include septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women resulting in spontaneous abortion (2^{nd} or 3^{rd} trimester), or a stillbirth. Gastrointestinal symptoms have been epidemiologically associated with use of antacids which significantly lower the infective dose.

Cryptosporidiosis: Many large-scale waterborne outbreaks have occurred in the United States. Particular attention is focused on the outbreaks in Milwaukee, WI and Carrollton, GA in which 400,000 and 17,000 persons, respectively, were infected. In another incident in Maine, a few hundred children were sickened by *Cryptosporidium*. The source was fresh-pressed apple cider made from apples gathered from a cow pasture (Millard et al., 1994). Conventional drinking water disinfectants such as chlorine and chlorine dioxide are not effective in killing *C. parvum*. The standard water treatment processes of coagulation, flocculation, and filtration are thought to be effective in removing this parasite when operating normally.

Giardiasis: Giardia lamblia: See Above

4.2.3 Effects of Pathogen Pollution

There is ample evidence that pathogens from agricultural operations have caused human disease outbreaks in the past. Ecological damage has also been indicated. Spread from animal to animal at the CAFO is a concern that individual operators have responded to with thorough periodic cleaning usually after one group of animals is sent to market and before another arrives.

Although more is known about the human diseases that may be caused by pathogens released from CAFOs, this section will also discuss the ecological effects of pathogens released into the environment.

An expert panel recently meeting on "Emerging Microbiological Food Safety Issues: Implications for Control in the 21st Century" concluded that control of manure has become a critical issue. Properly treated manure may be an effective and safe fertilizer, but untreated or improperly treated manure may contain pathogens that may reach fresh produce in the field or nearby water supplies.

The following text, tables, and references provide supporting evidence that farm animals held in CAFOS serve as an important reservoir for significant human pathogens and there are documented cases where serious disease outbreaks have occurred as a result of these animals' manure containing pathogens. Table 4.2 shows examples of manure-related human epidemics. A brief summary of each incident follows. These outbreaks involved *E. coli* O157:H7, *Campylobacter*, and *Cryptosporidium parvum*. All cases summarized below resulted in serious illness and even some deaths.

Table 4.2 Examples of Manure-Related Human Epidemics

LOCATION	YEAR	PATHOGEN	IMPACT	SUSPECTED SOURCE	REFERENCE
Walkerton, Canada	2000	*E. coli* O157:H7 & *Campylobacter* spp.	6 deaths, 2300 cases	Runoff from farm fields entering town's water supply	Valcour, J. E., et.al. *Emerg Inf Dis.*, March 2002
Washington Co, NY	1999	*E. coli* O157:H7 & *Campylobacter* spp.	2 deaths, 116 cases	runoff at fairgrounds	*Public Health Dispatch*, CDC, 1999
Carrollton, GA	1989	*Cryptosporidium parvum*	13,000 cases	Manure runoff	Solo-Gabriele, *JAWWA*, 88; 76-86
Swindon & Oxfordshire, UK	1989	*Cryptosporidium parvum*	516 excess cases	runoff from farm fields	Richardson, *Epidemiol. Infect* 107:485-495
Bradford, UK	1994	*Cryptosporidium parvum*	125 cases	storm runoff from farm fields	Atherton, *Epidemiol. Infect.* 115:123
Milwaukee, WI	1993	*Cryptosporidium parvum*	400,000 cases, 87 deaths	animal manure and/or human excrement	MacKenzie, *N. Eng. J. Med.* 331:161
Maine & Others	1993	*E. coli* 0157:H7	several illnesses	animal manure spread in apple orchard	Cieslak, *Lancet* 342:367
Sakai City, Japan	1995	*E. coli* 0157:H7	12,680 cases, 425 hospitalized, 3 dead	animal manure used in fields growing alfalfa sprouts	Fukushima, *Pediatrics International* 41:213
Cabool, MO	1990	*E. coli* 0157:H7	243 cases, 4 deaths	water line breaks in farm community	Geldreich, *Water Res.* 26:1127

4.2.4 Human Diseases: Examples of Manure-Related Human Epidemics, Case Studies of Problems and Potential for Problems with Pathogens in Animal Manure

4.2.4.1 Walkerton, Ontario

In May 2000, at Walkerton, Ontario, Canada, 2300 people were infected with *E. coli* O157:H7, and a smaller number were co-infected with *Campylobacter jejuni*. There were seven deaths, and more than 100 people were hospitalized. A direct link was made to cow manure as the source of the pathogens since a pasture occupied by cattle was located near the ground water source for the city's water supply.

4.2.4.2 Washington County Fair, New York

An outbreak of *Escherichia coli* O157:H7 and *Campylobacter* spp. also occurred among attendees of the Washington County Fair, New York in 1999. In this outbreak 116 cases were confirmed, 65 people were admitted to the hospital, 11 children developed HUS (Hemolytic Uremic Syndrome) and 2 children died. The link to cattle manure as the source was primarily through the isolation of these organisms from a shallow well on the fairgrounds and the knowledge that this organism is frequently found in cattle feces.

4.2.4.3 Carrolton, GA

In 1987 an estimated 13,000 people became infected with *Cryptosporidium parvum* due to a malfunction of the drinking water treatment plant. In addition to problems with the coagulation flocculation system, the filtration system was shut down periodically without backwashing the filters prior to each re-start. This failure of process control allowed *C. parvum* oocysts to freely pass through the filtration process. Carrolton, Ga. was the initial large-scale outbreak of crytosporidiosis in the United States.

4.2.4.4 Wilshire, Swindon, and Oxfordshire, England

An outbreak occurrd in Wilshire, Swindon, and Oxfordshire in January 1989, in which 516 cases were recognized, and 8% of the cases required hospitalization. The cause was traced to drinking water, and much emphasis was placed on the fact that the Thames River in this region drained cattle grazing areas. Extensive examination of the water treatment process was carried out, and a boil water order was issued. The outbreak(s) followed periods of heavy rainfall, and this factor supported the hypothesis that cattle manure was a source of the oocysts.

4.2.4.5 Bradford, England

In the community of Bradford, England, a city of 50,000 residents, 125 cases of cryptosporidiosis occurred over a 7-day period. All cases were confirmed by laboratory examination for oocysts. The average oocyst concentration in the city water supply was 0.019/L, and the outbreak occurred following a storm event in which excess water was draining from agricultural fields.

4.2.4.6 Milwaukee, Wisconsin

The largest waterborne outbreak of disease occurred March-April 1993 and resulted from a breach in treatment in one Milwaukee, Wisconsin water treatment plant. This event was responsible for 400,000 cases of illness and 87 deaths, with the deaths occurring among the immuno-compromised segment of the population. Both animal manure and material from a community wastewater treatment plant were implicated as likely causes of this epidemic.

4.2.4.7 Maine

There is evidence that a 1993 *E. coli* outbreak in Maine was the result of manure applications to a vegetable garden.

4.2.4.8 Sakai City, Japan

A massive outbreak of enterohemorrhagic *E. coli* O157:H7 infection occurred in July 1996 in Sakai City, Japan. The outbreak affected 12,680 school children and was caused by *E. coli* O157:H7. The pathogen was present in radish sprouts that the children consumed in a school lunch program. This is the largest outbreak due to this organism. From the original 12,680 children, 425 were treated at a local hospital, 121 developed the hemolytic-uremic syndrome. Three children died. This outbreak may be linked indirectly to cattle manure since the fields where the alfalfa sprouts were grown had been fertilized with manure.

4.2.4.9 Cabool, Missouri

In December 1989 and January 1990 contamination of the city water supply in Cabool, Missouri resulted in 243 cases of *Salmonella typhi* infection and resulted in 4 deaths. Cabool, MO is located in an agricultural area of Missouri with large populations of beef and dairy cattle in the region. The source of drinking water is ground water, and prior to the outbreak, chlorination was not part of the water treatment process. Additional manure related infectious disease outbreaks have been reported by [Morgan et al. (1998), Solomon et al. (2002)], and Gordeiko et al. (1990). Rather interestingly, two Q-Fever outbreaks related to manure were reported: one in Germany [Reintjes et al. (2000)] and one in England [Jorm et al. (1990)].

While the above summaries concern outbreaks of disease serious enough to involve the public health authorities, other diseases, though less serious, are more common. It was estimated in 1998 that 2-4 million persons were infected with some form of salmonella (USDA, 1994). Salmonellosis is characterized by flu-like symptoms, possibly accompanied by nausea, vomiting, abdominal cramps and diarrhea. Except for *Salmonella typhi,* which is exclusively a human disease, other forms of salmonellosis do not have high mortality rates but do have high morbidity rates and are highly transmittable. Major foodborne outbreaks have been related to consumption of beef, poultry, homemade ice cream, and pork (USDA, 1994). It may also be present in eggs. The incidence of salmonellosis appears to be rising both within the U. S. and in other industrialized nations. *S. enteriditis* isolations from humans have shown a dramatic rise in the past decade, particularly in the northeast United States (6-fold or more).

4.2.5 Animal Diseases

While the common pathogens may be a risk to humans, they are also a risk to other animals. Wild animals moving near manure application sites may carry diseases to new areas. Most ruminants, deer, elk, and others will probably be sensitive to the same organisms that affect cattle. Poultry diseases may also affect other birds. Geese and ducks are known to carry *Cryptosporidium* and *Giardia.*

Distribution of manure beyond the production facility bears the possibility of serious environmental and economic consequences, such as, for example, if there is an asymptomatic carrier of a disease. The manure from that farm could spread the disease to several other farms receiving manure as fertilizer. The consequences could range from increased veterinary bills to treat affected animals to wholesale destruction of infected animals, depending on the disease being spread. Biosecurity of farms has become an important issue with the USDA publishing several guidelines for farms to help secure production facilities from external contamination. Not all pathogens are present at every CAFO. Understanding the distribution of pathogenic organisms makes it easier to design control strategies that will reduce risk. Table 4.3 shows the sources of common zoonotic diseases on farms as a function of livestock species (Cole, 1999).

Reservoirs for *Yersinia enterocolitica* include most domestic mammals, particularly swine. Reservoirs for *Yersinia pseudotuberculosis* include a wide variety of domestic mammals and fowl. The recently discovered hemorrhagic colitis strains of *Escherichia coli* belonging to the O157:H7 serotype are usually acquired after ingestion of either rare ground beef or raw milk. They have also been shown to be transmitted via water. These verotoxin-producing (shigatoxin) strains have been isolated from calves and pigs with enteric diseases and from retail pork and lamb. Reservoirs of *Campylobacter jejuni* include cattle, sheep, swine, dogs, and domestic poultry (USEPA, 1998). Both *E. coli* O157:H7 and *Salmonella* spp. are carried by ruminants, especially cattle, and at least one to five percent of cattle shed *E. coli* O157:H7 in feces. (Altekruse et al., 1997; Hosek et al., 1997).

Table 4.3 Sources of common zoonotic diseases on farms.

	Poultry			Swine	Cattle	
Pathogen	Broilers	Turkeys	Layers		Dairy	Beef
Listeria Monocytogenes					▲	▲
Crytosporidium parvum					▲	▲
Giardia lamblia					▲	▲
Salmonella sp.	▲	▲	▲	▲	▲	▲
Pathogenic *E. coli*					▲	▲
Yersinia enterocolitica				▲		
Leptospira sp.				▲	▲	▲
Campylobacter sp.	▲	▲	▲	▲	▲	▲
Brucella sp.				▲	▲	▲
Erysipelothrix rhusiopathiae		▲		▲		

The gram positive bacterium *Listeria monocytogenes* is widely distributed in the environment and is associated with decaying vegetation, soil, sewage, and feces of animals. Many cases of human listeriosis have been associated with consumption of fresh vegetables possibly contaminated with manure from ruminant animals. *L. monocytogenes* may grow on a variety of vegetables even at refrigeration temperatures. (Brackett, 1999) Therefore, the potential for introduction and transmission of *L. monocytogenes* from manure and soil amended with raw or poorly treated manure on produce may be greater than vegetables grown in soil amended with treated manure.

4.3 Antibiotics

Antibiotics are used extensively in animal production. Approximately 2.5 million kilograms of antibiotics per year are used on livestock in the United States (Kolpin et al., 2000). Of this amount, about 10% is used to treat active infections while the remaining nearly 90% is used for growth promotion and prophylactic care.

Antibiotics may be beneficial in agriculture, but there are growing concerns about the effects of antibiotics in the environment, especially the possibility of the increase in populations of drug-resistant microbes. An increase in drug resistant microbes could make it more difficult to treat diseases in animals and humans. Almost 50% of the antimicrobial agents in North America are used by agriculture. The majority of agricultural use is for growth promotion in farm animals. Growth promotion uses low doses of antibiotics that may lead to more bacterial resistance than higher doses used therapeutically (McGreer, 1998).

Antibiotic residue may be found in animal by-products (manure and urine). This waste may come in contact with humans, other animals, and surface and sub-surface waters through run-off and leaching. The concentrated use of antibiotics at CAFOs makes it more likely to have antibiotic residue and antibiotic resistant microbes in the vicinity.

Wide use of antibiotics may lead to development of resistance among the microorganisms that the antibiotics are being used to control. Antibiotic resistance develops in microbial populations due to the selective pressure exerted on the population by the antibiotic. If the level of antibiotic used is inadequate to completely eliminate the microorganisms from the animals some members of the population will survive. These organisms will continue to increase their resistance to the antibiotic until the antibiotics are no longer effective in controlling populations or diseases. The enzymatic capacity for resistance to antibiotics may be transferred in the environment by different mechanisms. Plasmids may be transferred directly from microorganism to microorganism, by bacteriophages, or upon cell lysis, leading to the uptake of free plasmids by other organisms. Increasing microbial resistance to antibiotics raises the possibility of hard-to-control animal sickness and require use of multiple antibiotics for treatment. Microbes could then become resistant to multiple antibiotics. Since the antibiotics may also be spread throughout the environment via manure and urine, other microbes that come into contact may also become resistant. This includes not only microbes that lead to animal diseases but to human maladies as well. Since the antibiotics used for animals are often the same for humans, different antibiotics may have to be used to fight the resistant microbes. One possibility to prevent this particular problem would be to limit the use of "human" antibiotics on animals.

4.3.1 Case studies on the effect of antibiotics related to CAFOs on the environment:

4.3.1.1 Case 1 – Chesapeake Bay

In the Chesapeake Bay area, manure from a chicken CAFO was used to fertilize fields. The runoff from these fields fed into the Pocomoke River changing the ecology of the river. Recently an outbreak of *Pfiesteria piscicida*, which is toxic to fish and human health, was attributed to the influx of antibiotics from the field runoff. A study has shown that this strain of *Pfiesteria piscicida* found in the Pocomoke River is antibiotic resistant whereas other strains from similar rivers do not show the same antibiotic resistances (Isbister et al., 2000).

4.3.1.2 Case 2 – Iowa Swine Operations

A study conducted by the Iowa Department of Public Health on the effects of CAFOs on the environment showed the presence of antibiotics and antibiotic-resistant microbes in the earthen manure lagoons. The tests revealed an antibiotic in an earthen manure lagoon monitoring well. Four different antibiotics (tetracyclines, sulfonamides, β-lactams, and macrolides) were found in detectable concentrations (Table 4.4).

Table 4.4. Antibiotic Levels in the Lagoons and one Monitoring Well (adapted from Table 7) (Iowa Dept. Public Health, 1998)

Collection Sites (Farm)	Tetracycline (µg/L)	Sulfonamide (µg/L)	β-Lactam (µg/L)	Macrolide (µg/L)
Lagoon (1)	250	>20	<2	227
Lagoon (2)	11	>20	<2	<10
Lagoon (3)	150	>20	<2	60
Lagoon (4)	68	>20	3.5	<10
Lagoon (5)	66	>20	2.1	81
Lagoon (7)	540	>20	2.1	275
Lagoon (8)	110	>20	2.9	15
Monitoring Well (8)	<1	7.6	<2	<10

E. coli, Enteroccus, and, *Salmonella* were obtained from the lagoons, wells, and drainage ditches on the sites. All these microbes showed varying antibiotic resistance (Iowa Dept. Public Health, 1998).

4.3.1.3 Case 3 – Shoal Creek

Researchers studying bacteria in Shoal Creek, located in Barry County, Missouri, found detectable concentrations of antibiotics in the creek. This northwest section of the county produces 33 million broiler chickens and 300,000 turkeys annually. The antibiotic source was found to be a chicken CAFO located upstream from where the antibiotics were found. Antibiotics used to treat both animals and humans as well as human only (located downstream of sewage plant effluents) were also found. Further study on the impact of the antibiotics to the watershed and ecological structure of Shoal Creek is on-going (Penprase, 2001).

4.3.1.4 Case 4 – A National Reconnaissance

The U.S. Geological Survey tested water samples from 139 streams in 30 states in 1999 and 2000. The selection of sampling sites was biased toward streams susceptible to contamination (i.e., downstream of intense urbanization and livestock production). The samples were tested for pharmaceuticals, hormones, and other organic wastewater contaminants. Of the 95 organic wastewater contaminants tested, approximately 20 antibiotics were measured and only eight were not found in the samples (however, some of them may have been present in the stream sediment due to "their apparent affinity for sorption to sediment."

Figure 4.3 shows the frequency of detection and percent of total measured concentration for the contaminants, by category (Kolpin, et al. 2002).

The widespread use of antibiotics in agriculture, especially CAFOs, is now becoming an area of investigation in the United States.

4.4 Endocrine Disrupting Chemicals Associated with Concentrated Animal Feeding Operations

Endocrine disruptors are a class of chemicals of growing interest to the environmental community. The U.S. Environmental Protection Agency's (EPA) Risk Assessment Forum defined an endocrine disrupting chemical (EDC) as "an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior (EPA 1997) ". Most of us are more familiar with chemicals of concern that have a specific health outcome such as lung cancer. However, EDCs are a class of chemicals defined by their mode of action and may result in a variety of health outcomes. For example, an EDC may initiate a health-related outcome in humans or wildlife by binding to and stimulating estrogen or androgen receptors.

Steroid hormones are chemicals of concern to endocrine health associated with CAFOs. Steroid hormones are used by many animals to facilitate the control of their body systems. Mammals, birds, reptiles, and fish produce virtually the same steroid hormones and possess receptors that bind the steroids to receive their control messages (McLachlan 2001). In this section, the term hormones will refer to steroid hormones. Until risk assessments are completed, it is assumed that all endocrine active compounds that have the potential to interact with the environment are chemicals of concern. Thus, the chemicals of concern are those hormones naturally produced and excreted by animals and those hormones administered to animals as drugs and are excreted. These animals remove hormones from their bodies by excreting them

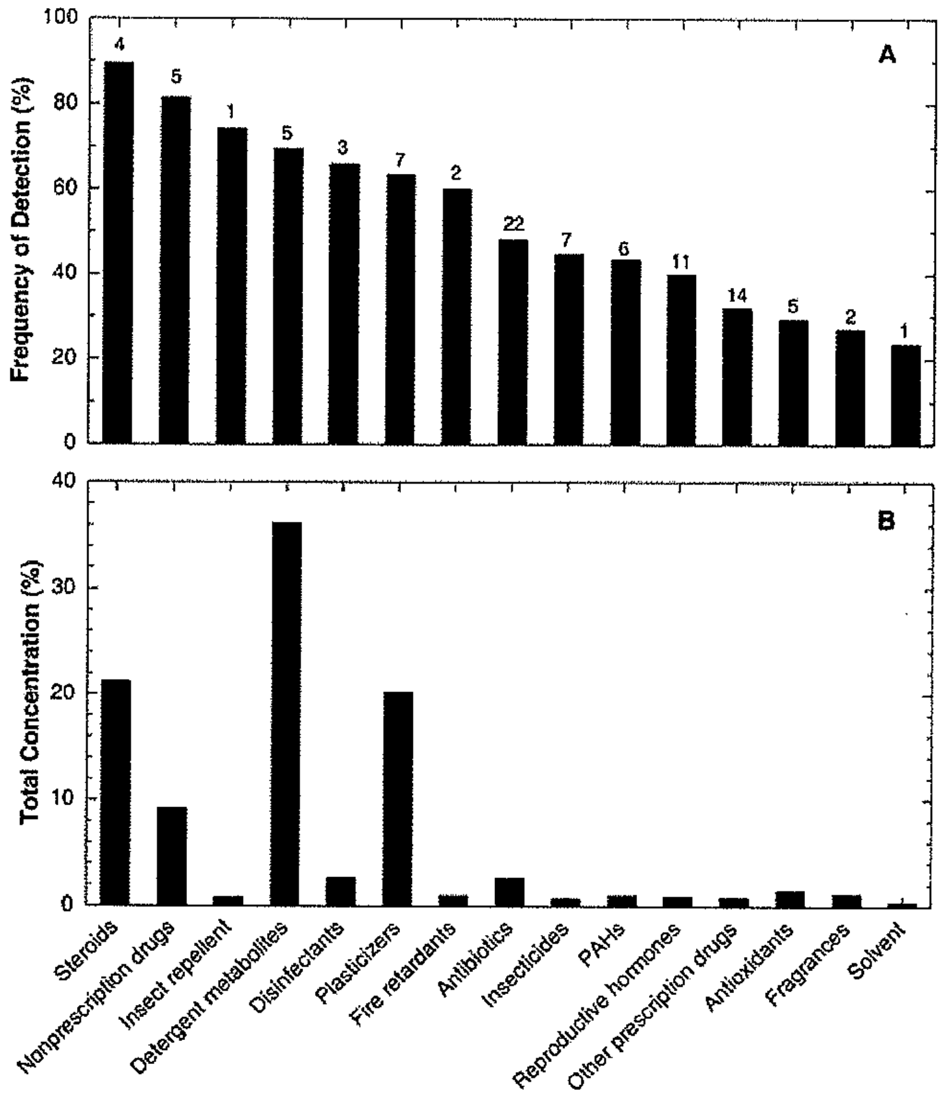

Figure 4.3. Frequency of detection of organic wastewater contaminants by general use category (4A), and percent of total measured concentration of organic wastewater contaminants by general use category (4B). Number of compounds in each category shown above bar (Kolpin, et al., 2002).

in urine or feces. Many of the methods of storage, treatment, and disposal of animal wastes at CAFOs allow contact of the waste with the environment. Since many animal species respond to the same hormones, it may be possible to disrupt the natural state of the endocrine systems in wildlife exposed to waste from CAFOs. If CAFO-generated hormones are transported to water bodies (surface or ground water), exposure to humans may be possible.

The classes of natural (biogenic) hormones that may be excreted by animals include estrogens, androgen, progesterones, and thyroid hormones. Although ideally all hormones would be considered in this risk management evaluation, there is almost no information available about natural hormones and animal feeding operations other than estrogens and, to a lesser extent, androgens. There is no information available on CAFOS and thyroid hormones. Thus, the focus of this section will be on natural estrogens and veterinary hormones.

The chemical structures of the primary natural estrogens are shown in Figure 4.4. Here, they are shown in their biologically-active forms. Generally, hormones the body wishes to excrete are conjugated with glucoronides or sulfonides. Conjugation eliminates their biological activity and increases their solubility in water. Most literature concludes that excreted, conjugated hormones are deconjugated relatively quickly in the environment by enzymes produced by common bacteria (Schiffer, Daxenberger et al. 2001). It will be assumed that hormones in contact with the environment are not conjugated. The most active estrogen is 17β estradiol, while estrone and estriol are metabolites of estradiol with much less biological activity.

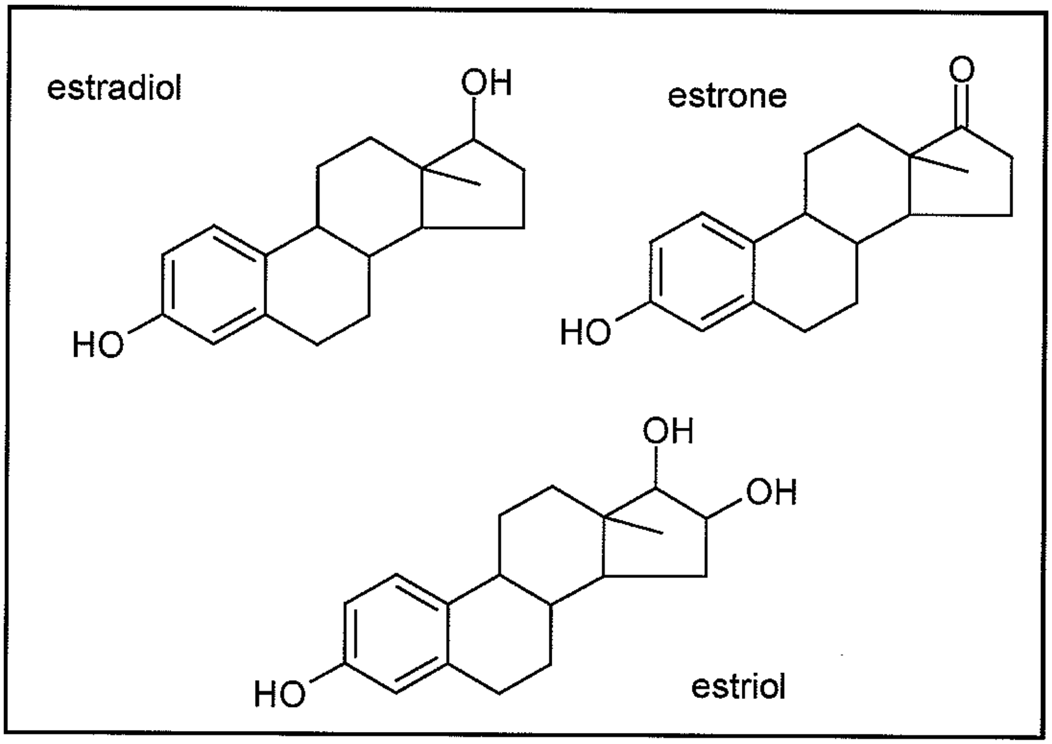

Figure 4.4. Structure of biogenic hormones.

4.4.1 Xenobiotic Hormones

The U.S. Food and Drug Administration (FDA) has approved the veterinary use of the six hormones (Table 1) and only for cattle and sheep (21 CFR, Chapter 1, Part 522). Patented forms of the natural hormones are often used in cattle and sheep production. These include estradiol benzoate (17β-estradiol 3-benzoate) and estradiol valerate (17β-estradiol 17-pentanoate), testosterone propionate, and various derivatives of progesterone, generically called progestins. Xenobiotic hormones administered to cattle and sheep include trenbolone acetate (TbA), melengestrol acetate (MGA), and zeranol. Zeranol is an estrogen mimic. TbA is hydrolyzed *in vivo* to the biologically active chemical, trenbolone-17β (TbOH-17β) (Schiffer, Daxenberger et al. 2001). TbOH-17β acts as an androgen, and an antiglucocorticoid. TbOH -17β

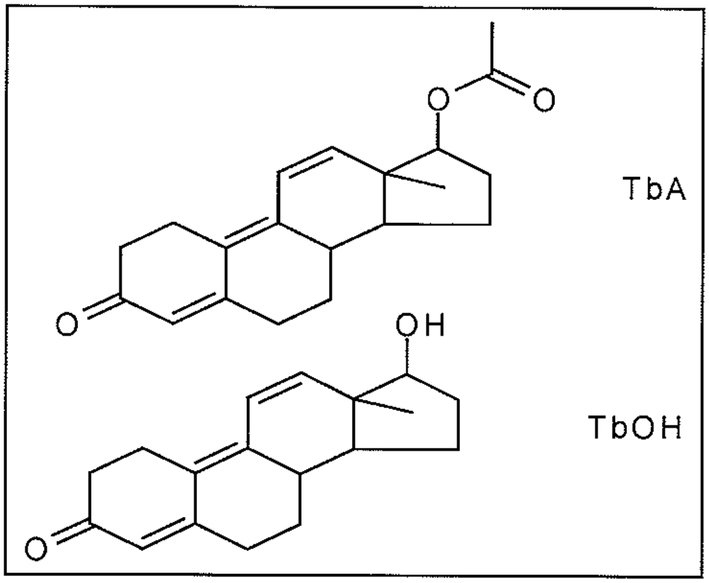

Figure 4.5. Chemical structure of Trenbolone acetate and hydroxide.

may be metabolized to TbOH-17αε which is 40 times less active than TbOH- 17β. Zeranol is an estrogen mimic. The chemical structures of these compounds are shown in Figure 4.5.

MGA is used for estrus synchronization or induction to improve feed efficiency and weight gain in heifers (Schiffer, Daxenberger et al. 2001). MGA acts as a progesterone and glucocorticoid.

The parent veterinary drug, trenbelone acetate (TbA), is metabolized to the biologically active chemical, trenbelone-17β (TbOH-17β) and TbOH-17α. The β and α are isomers where the methyl and hydroxyl groups are *cis* and *trans*, respectively.

Since steroid hormones are the signal molecules of the endocrine system, organisms exposed to these hormones have the potential for adverse endocrine related effects. The consequences of excess estrogen in humans may be dramatic (Williams Textbook, 1998) and effects at low doses are possible (Anderson, 1999). Unintentional exposure of wildlife to estrogens has focused mostly on fish: vitellogenin production in male fish has been observed when exposed as little as 1 ng/l 17β estradiol or 25 ng/l estrone (Routledge, 1998). Other estrogen-related health effects observed in wildlife include abnormalities in reproductive organ development and sex change. *In vitro* assays that measure binding to human steroid receptors have shown that TbOH -17β binds to the human androgen receptor as strongly as the natural human androgen, dihydrotestosterone, and MGA binds 3.5-times stronger to the human progesterone receptor than progesterone itself (Bauer, Daxenberger et al. 2000).

4.4.2 Uses of Hormones in CAFOs

Farm animals generate, use, metabolize, and excrete natural hormones, the type and quantity depending on the animal, sex, and reproductive state.

The FDA has approved the veterinary use for cattle of the hormones listed in Table 4.5 in single hormone or dual hormone doses (21 CFR, Chapter 1, Part 522). The delivery of the hormones is typically

Table 4.5. Hormones Approved for Veterinary Use in Cattle

Hormone	Biological Activity	Commercial Forms
17β-estradiol	estrogenic	estradiol benzoate, estrodiol valerate
testosterone	androgenic	testosterone propionate
progesterone	progesteronic	progestin
trenbelone acetate	androgenic	same
melengestrol acetate	progesteronic	same
zeranol	estrogenic	same

accomplished by ear implant (although delivery of MGA in feed is approved by the FDA). The FDA has approved several dual hormone implants, including an implant containing 20 mg TbA and an implant containing 20 mg estradiol benzoate with 200 mg testosterone propionate. Data on the rate of use of these hormones in the United States were not found.

Arcand-Hoy *et al.* (Arcand-Hoy, Nimrod et al. 1998) estimated the use of exogenous estradiol (presumably the sum of the use of simple estradiol and the benzoate and valerate forms) to farm animals to be 580 kg/yr in the United States.

4.4.3 Release of Hormones to the Environment

Since hormones are present in animal excreted waste and in their bodies, excreted waste (urine and feces) and animal carcasses that come into contact with the environment must be considered as likely sources of hormones to the environment. Although the hormone content of waste has not been systematically studied, a relatively large total mass of hormones is released yearly given the estimated 291 billion pounds of manure generated annually in the United States (EPA 2001). The avenues of release of animal waste into the environment at CAFOs are described in detail in other sections of this RME. These releases may be associated with leakage from storage lagoons, runoff from composting operations, land application of waste, and other scenarios. There are very little data to quantify the release rates of hormones to the environment from CAFOs. One study found that chicken litter may contain > 100μg/kg estrogen and that runoff from a field receiving poultry waste contained up to 3.5 μg/l estradiol (Shore, Cornell et al. 1995). A similar study found 1.3 μg/l estradiol in runoff from land applied with poultry waste (litter) (Nichols, Daniel et al. 1997). Testosterone was found in rooster litter up to 670 μg/kg (Shore, Harel-Markowitz et al. 1993). In another study, MGA and metabolites of TbA were measured in the dung of cattle given implants of MGA or TbA (Schiffer, Daxenberger et al. 2001). The maximum levels found in the dung were 7.8, 75, 4.3 μg/kg of MGA, TbOH-17α, and TbOH -17β, respectively. Although there is little data, the U.S. EPA acknowledges that hormones should be considered in assessing the environmental impact of CAFOs (EPA 2001).

A recent news article quoted as yet unpublished work by U.S. EPA and university researchers regarding a study of the hormonal character of a stream associated with a cattle feedlot in Nebraska (Raloff, 2002). The research found that water collected downstream of the feedlot had significantly higher androgenic activity than water collected upstream.

4.5 Metals

4.5.1 Use of Metals in Animal Feed

Animals in CAFOS produce a great amount of manure that is applied to land as fertilizer. The metal content of animal waste is in question. Metals are being supplied to farm animals via diet. This review of the literature investigates the disbursement of the nutrient-rich excreta and the effects that are or may be encountered.

Metals in discussion here are copper, zinc and arsenic. While trace amounts of some elements are necessary for life, quantities above and beyond those amounts are fed to swine and poultry as growth promoters. Usually arsenic (often in the form of "roxarsone", Christen, 2001) is fed to chickens for this purpose, even though arsenic is not a required nutrient; exaggerated amounts of copper and zinc (often in the form of $CuSO_4$ and ZnO or $ZnSO_4$, respectively) are typically used in the swine diets. Possible adverse effects reported in the literature include the risk of phytotoxicity, groundwater contamination, and deposition in river sediment that may eventually release to pollute the water, the effect of manure application on grazing animals and also the result of using chicken litter for livestock feed.

The use of excess metals to promote growth is practiced in many countries. For example, Canada (DeLange, 1997), Great Britain (Nicholson, 1999), Japan (Eneji, 2001), France (Martinez, 2000), Germany (Rothe, 1994), Spain (Alonzo, 2000), Denmark (Tom-Petersen, 2001) and others have engaged in research to address issues similar to those of concern in the United States. Though the study parameters and methods of research may differ, overall, there are questions and conclusions that are nevertheless relevant to the demands of this discussion and are therefore taken into consideration.

The following table (Table 4.6) presents dietary/manure content data to give the reader an idea of the amounts of copper and zinc consumed by pigs when fed diets that achieve normal growth and those that promote growth. Arsenic is not a dietary requirement for poultry, the growth promoting level 5-10 ppm yields manure with 15-45 ppm (Muller, 2002; Chaney, 2002; Alonso, 2000; Ohio State Univ Bulletin, 1998).

Table 4.6 Copper and zinc in swine diets

Swine Diets (ppm)	Required Cu	High Cu	Required Zn	High Zn
Weanling/piglet	6	125-250	80-100	2000-3000
Manure (ppm)	~5.4	~113-225	~72-90	~1800-2700

4.5.2 Mobility of metals in soil

Mobility of the excreted metals has been addressed by some sources. Martinez (2000) examined the copper and zinc balances in soil after five years of repeated pig slurry applications. The results showed that most of the nutrient copper and zinc (80% of what was applied) remains in the top 0-20 cm of the soil layer. Tables 4.7 and 4.8 show soil analysis data for copper and zinc.

Table 4.7 Soil Cu balance after five years of repeated pig slurry application.

Soil Layer	Soil Cu content, mg/kg		EDTA Cu kg/ha		Increase in soil Cu	Recovery of Cu from slurry applied
	1991	1996	1991	1996		
0-20	2.8±0.3	35.1±2.8	7.3	91.3	84	45.8
20-40	2.2±0.5	12.6±2.6	5.6	32.8	27.2	14.8
40-60	1.3±0.3	2.2±0.7	3.3	5.6	2.3	1.3
Total	----------	----------	16.2	129.7	113.5	61.9

Table 4.8 Soil Zn balance after five years of repeated pig slurry application.

Soil Layer	Soil Zn content, mg/kg		EDTA Zn kg/ha		Increase in soil Zn	Recovery of Zn from slurry applied
	1991	1996	1991	1996		
0-20	2.7±0.8	64.1±5.8	6.9	167.0	160.1	60.2
20-40	2.3±0.7	15.4±2.4	5.9	40.0	34.1	12.8
40-60	0.8±0.3	1.8±0.5	2.1	4.6	2.5	0.9
Total	----------	----------	14.9	211.6	196.7	73.9

Gettier et al., (1988) is in agreement by finding that copper, when applied to the soil surface via pig manure, shows little movement through the soil profile (i.e., 0 – 20 cm) used in that experiment. (Also, see Table 4, data from World Animal Science, 1987.) It was reported that copper applied to soil generally results in a linear increase in extractable copper. Similarly, Mohanna et al., (1999) found a linear relationship between dietary zinc supplementation and the amount excreted. It has been advised that, with pig slurry application, immediate effects may not be recognized. Because metals may accumulate in the topsoil, it may be the longer term applications that reveal adverse effects (i.e., changes in soil biomass and herbage metal concentration) (Christie et al., 1989 and Eneji et al., 2001).

During an eight year period, Martinez et al. (2000), assessed the copper and zinc content of soil and drainage water in soil subjected to intensive pig slurry application. About 62% of the applied copper and 74% of the applied zinc remained in the soil as EDTA extractable forms. Only 0.05% and 0.6%, respectively, were present in the drainage water. A study of 18 soils in the Netherlands reported information concerning the correlation of organic matter and desorption of several metals (Impellitteri, et al., 2002). The results indicated that increasing pH increased soluble organic matter and Cu. Increased Ca flocculated organic matter and restrained Cu in solution. McBride (1994) stated that Cu added to soil will remain there for very long times. High organic matter increases mobility, but Cu is least soluble at pH near seven. Zinc may leach to lower levels of the soil if there are significant inputs of Ca to displace it from the exchange sites. Arsenic behaves in soil much like phosphate. That is, As only moves lower in the soil profile if the sorption capacity of the upper layers is filled. Based on this information, the amount of metals in the drainage may be very small, but some of the excreted metals do get carried into the groundwater, eventually making their way to a stream or river bed. Here, strewn about the sediment, the metals may be set into motion again as environmental conditions change (e.g., pH, redox potential, or high stream flow) (Lim et al., 1995). At that point, environmental consequences may become severe, even if they are delayed in time from the point of initial contamination. As, Cu, and Zn all have potential toxicity to plants and animals. Arsenic is found in soils from about 3.6 to 8.8 mg/kg. Copper is found in soils from about 14 to 29 mg/kg. And zinc is found in soils from about 34 to 84 mg/kg. Copper and zinc are both essential elements for plant and animal life. The needed levels and the toxic levels will change as environmental conditions change. Arsenic is not an essential element and is more toxic to plants and animals than Cu or Zn.

4.5.3 Metals in plants

Christie et al. (1989), conducted a sixteen year study addressing herbage concentrations of copper and zinc that had reached 10 and 44 mg/kg, respectively. The purpose was to determine the toxicity to grazing sheep. See Table 4.9 for content data. While a very high rate of application (200 m^3/ha/yr) was used to test extreme conditions, this rate produced enough soil copper and zinc accumulation sufficient to produce a toxic response from sheep (>10 mg/kg). Sheep are especially susceptible to excess copper, and a prolonged ingestion of just 15 - 20 ppm of copper may result in the occurrence of fatal hemolytic crisis. (World Animal Science, 1987)

Table 4.9 Cu and Zn in soil (top 5 cm), February, 1987 and herbage first cut of 1986.

		Copper				Zinc			
Treatment	pH	EDTA extract	Total	Extractable/total	Herbage	EDTA extract	Total	Extractable/total	Herbage
Fertilizer	5.1	4.8	14.8	0.32	3.9	4.4	55.9	0.08	13.5
Control	5.4	5.4	15.5	0.35	3.6	4.1	58.0	0.07	14.3
Pig Slurry, m^3/ha/yr									
50	5.6	25.9	40.2	0.65	4.6	15.2	76.6	0.20	21.5
100	5.3	49.6	69.0	0.72	8.2	26.4	93.9	0.29	34.0
200	5.1	85.2	110.8	0.77	10.1	50.8	110.8	0.46	43.7
Cow Slurry, m^3/ha/yr									
50	5.8	6.4	16.3	0.40	3.3	6.6	61.1	0.11	13.2
100	6.0	7.7	19.0	0.41	4.2	9.8	69.4	0.14	14.3
200	6.2	9.6	21.6	0.45	7.2	16.0	76.8	0.21	20.5
L.S.D. at 5% level	0.1	5.7	5.1	0.06	1.0	3.9	8.5	0.04	3.5

All values are mg/kg dry material. L.S.D. least significant difference (minimum difference to have significance).

While some studies proposed a threat of copper and zinc phytotoxicity, there was not an abundance of conclusive data. Tom-Petersen, 2001, explained that if the accumulation of copper in the soil reaches a toxic level, structure and function of the microbial community may be affected. But this source goes on to say that a lack of knowledge on the interaction between copper and the biota makes it difficult to assess the impact on a biological system. Likewise, Gettier, et al., 1988 states that while a high level of copper in soil is phytotoxic, the amount of copper that may may safely be added to a soil system has not been well defined. World Animal Science, 1987, has indicated that zinc is partly added to high copper diets to counteract the accumulation of copper in animal tissue, and accumulation of either of these metals in soil could cause phytotoxicity in which the plant root system is affected first.

4.5.4 Metals in Animals

Alonso, et al., 2000, performed a study to determine whether pig slurry treated fields have an effect on the accumulation of copper and zinc in grazing cattle. It has been suggested that ruminants may be more at risk for copper toxicity because of their efficiency in absorbing trace elements across the gut, which may lead to toxic levels of copper in the liver. When the liver reaches saturation with copper, the copper is released quickly into the blood. In sheep, copper may cause fatal hemolytic crisis. This study concluded that, in areas with the highest pig densities, more than 20% of the cattle examined had hepatic copper levels exceeding the toxic concentration of 150 mg/kg fresh weight. Zinc liver levels, however, did not seem to be of any consequence.

The use of chicken litter (consisting of poultry manure, feathers, bedding, and spilled feed (Poore, et al., 1998) as livestock feed is yet another area of concern. While broiler litter has been used for over fifty years with no major problems, research performed at Virginia Tech. reported increases in arsenic and copper concentrations in the livers of cattle fed poultry litter. The arsenic concentration, however, returns to control levels within three days of withdrawal. Therefore, most states recommend a fifteen-day withdrawal period prior to slaughter. A related study indicated that while increased liver copper concentrations in cattle fed poultry litter without adverse effects have been reported, it was found in this study that the feeding of 1.13 kg $CuSO_4$/90.7 kg chicken litter to cattle resulted in chronic copper toxicosis. However, this condition may be reduced by supplementation of molybdenum and thiosulfate (Banton, et al., 1987). For reference, the level of arsenic in a typical chicken manure/litter is about 25 ppm. (Chaney, et al., 2000) A literature search for additional information regarding the role of arsenic contamination of soil via chicken manure (e.g., phytotoxicity, drainage water, sediment residue, etc.) was not fruitful.

Metals are excreted in various forms by animals. A common form of copper is the divalent ion that may form complexes with organic matter. Similarly, zinc has a divalent form that will also complex with organic matter. Arsenic more closely resembles phosphorus in its behavior.

4.5.5 Summary

The following comments are extracted from this review of dietary copper, zinc, and arsenic consumption by pigs and poultry and the distribution of these metals when excreted.

1. Copper and zinc are fed to swine in concentrations that exceed the minimum requirements to induce a growth promoting effect. In chickens, arsenic is used as a supplement for growth promotion; arsenic is not a dietary requirement in chicken feed.

2. Approximately 80-90% of the copper, zinc, and arsenic consumed is excreted.

3. Most of the excreted metals, contained in manure/slurry for land application, settle in the topsoil, approximately the first 0 - 20 cm of soil.

4. World Animal Science, 1987, reports that pig manure slurry, on the average, contains six times more copper than either poultry or cattle slurry. This presents a more striking danger of copper enrichment in those soils being fertilized with pig excreta.

5. Zinc added to a high copper diet helps thwart the possibility of copper toxicity.

6. In swine, the response to feed additives is greatest in starter diets (10-50 pounds). Higher levels of copper and zinc are typically found in the diet at this level. (KSU, October 1997)

7. A management plan needs to be established for each CAFO, on an individual basis, that takes into account variables such as soil type, soil pH, land area for manure application, level of waste water produced, animal density, anticipated metal output, etc.

8. There is a need to identify other growth promoters that would be non-toxic, or at least identify other forms of the metal compounds being used now that would be more bioavailable. For example, cupric citrate was found to promote growth at lower levels than cupric sulfate pentahydrate, resulting in less litter copper (Pesti, et al., 1996).